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Mucous Membrane of Respiratory Epithelium

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Of the eight epithelial cell types of the airway surface epithelium three are secretory, the mucous, serous and Clara cells: the first two are also found in the submucosal glands. Organ culture results indicate that the pattern of control of the surface cells is different from that of the glands. Our recent studies show that in the surface epithelium Clara and serous cells can quickly convert to mucous as do nonsecretory undifferentiated cells. The balance between the various cell types changes with airway level.

The type of glycoprotein within the secretory granules is neutral or acid, sialylated or sulfated, and also shows a regional pattern. Homeostasis is maintained in the normal but the equilibrium is quickly upset by a variety of irritants, infection or drugs. Change in pattern of glycoprotein synthesis depends largely on change of granules. The granules at the cell apex change first. The nature and distribution of the various receptor binding sites is significant in patterns of control. This lability occurs in an intact epithelium and whether or not mitotic activity is increased. With irritation tolerance to stimulus develops. Anti-inflammatory agents can protect against some of these cellular and intracellular events. Our organ culture studies and biochemical analysis of secretion complement the tissue studies.

Recent studies show that isoproterenol and salbutamol (a nonselective β agonist and a selective β_2, respectively) alter the normal mix of cell types and quickly, but that they have different regional specificity.

Although there are animal models in which the airway damage is associated with necrosis and sloughing of epithelium, this report concentrates on animal models in which the nature and dose of the damaging agent are such that the epithelium remains intact. Such models are especially relevant to understanding the effect of a relatively low dose of irritants whether delivered in acute or chronic exposure. It is a remodelling of the cell population of the normal intact epithelium that occurs, one that is particularly related to mucus hypersecretion, doubtless the most obvious single feature of the response and almost certainly the most important functionally (*1, 2*).

Even when the injury is serious enough to cause ulceration we have shown healing occurs while the administration of the irritant continues and in the new epithelium a similar remodelling is seen to that discussed below (*3*). Our most recent studies show that changes that we identify with chronic irritation occur quickly, much more quickly than previously suspected (*4, 5*).

In such studies it is essential to use animals with "clean" lungs (*6, 7*). The normal human airways are sterile, and even with mucus hypersecretion this state often continues. We find that histological criteria are more sensitive to determine satisfactory cleanness than bacteriological or viral studies. Lymphocyte infiltration may be of an unacceptable degree even when the lungs are sterile.

The mixed nature of the mucus or total bronchial liquid, and the variety of epithelial cells responsible for the special mucus or epithelial glycoproteins (*8-10*) call for a multidisciplinary approach. Animal models or *in vivo* studies with which we are particularly concerned here can be complemented by *in vitro* studies of the tissue cells. (*11-13*). Histochemical studies (*14-19*) are complemented by biochemical analysis of total secretion, both its broncial and serum components (*20-24*). These in turn need to be correlated with rheological behavior (*23-25*).

Cells of the Respiratory Epithelium

Within the airway epithelial lining eight epithelial cell types have now been identified (*8, 9*) (Fig. 1). All cells touch the basement membrane, only two do not

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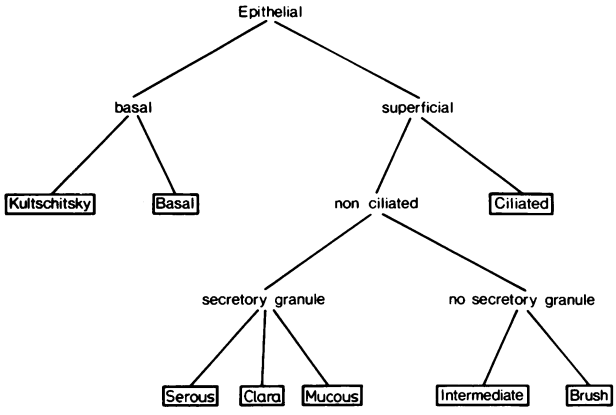

FIGURE 1. Eight epithelial cell types present in rat airway epithelium.

reach the lumen—the basal and Kulschitsky cells. The serous, Clara and mucous are the secretory cell types. The undifferentiated or intermediate cell, the ciliated and brush cells are not secretory. "Mucous" is the term we now prefer to goblet cell, since we can define it by reference to the nature of the secretory granules, whereas "goblet" refers essentially to the amount and shape of the secretory mass which resembles a chalice or goblet. The serous and Clara cells are each characterized by electron-dense and discrete granules: the Clara cell has a considerable amount of smooth endoplasmic reticulum and its apex often bulges above the tight junctions with neighbouring cells. Two mesenchymal cells are found: the globule leucocyte and the lymphocyte.

Reconstruction of the human submucosal gland reveals a duct about 1 mm long, lined by the duct cell — a tall cell, packed with mitochondria and reminiscent of the striated duct of the salivary gland, save that it lacks the striations (*26*). The tubules are lined by mucous or serous cells, the "serous" regions being always distal to a "mucous" region, i.e., further from airway lumen, and arising either as a lateral pouch or from the distal ends of a mucous tubule (Fig. 2) (*27*).

Nerve fibers are found within the airway epithelium (*28*). In the rat they are nonmyelinated and only in the extrapulmonary airways. They are numerous, particularly in the young animals, and are closely associated with basal secretory and ciliated cells. The nature of the vesicles they contain suggests that some are sensory, some motor, and of the latter, some adrenergic, some cholinergic. Nerve fibers are found within the basement membrane of the submucosal glands. Myoepithelial cells and "clear" cells, probably immunoblasts, are also found in the secretory tubules of the gland.

In the rat, mitotic activity represents turnover of the airway epithelium that is, in some regions, as fast as 10 days and in others 250. This range takes into account all regions, male and female, and three ages (*6*) (Fig. 3).

Normal Homeostasis

Under basal conditions, homeostasis of the airway epithelium is maintained with regard to several of its features, the normal pattern varying from region to region. The nature of the control mechanisms is not understood. Homeostasis is maintained with respect to the cell types present. For example, in the trachea the basal cell is numerous, making up about 30% of the cell population, whereas it is virtually absent in peripheral airways; the Clara cell, the secretory cell of small airways, is not seen in the trachea. Of the cells that are present, the proportion varies at vari-

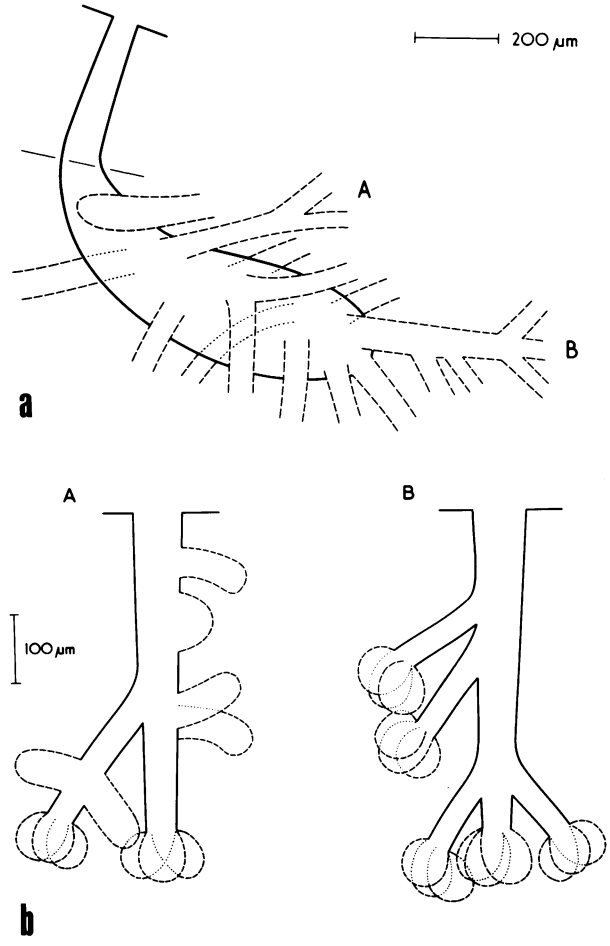

FIGURE 2. Reconstruction of human bronchial gland drawn to scale, showing collecting duct with tubules arising from it. The branching patterns of two tubules arising from the duct are shown: (——) mucous cells; (- -) serous cells. Secretion from the serous cell thus passes over the mucous cell before reaching the collecting duct.

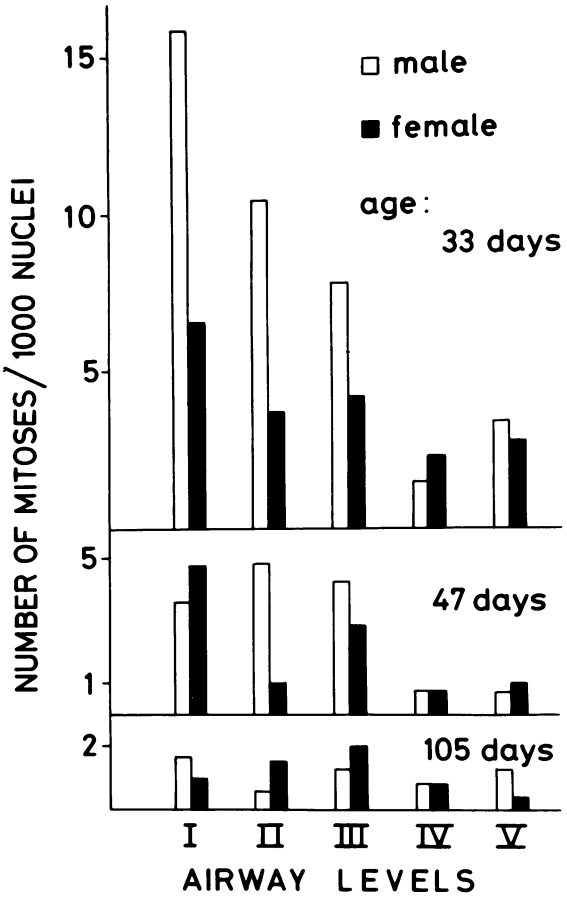

FIGURE 3. Mitotic index (number of mitotic nuclei per 1000 nuclei) in male and female rats of three age groups and at five airway levels: (levels I-III) proximal, mid- and distal trachea; (level IV) main intrapulmonary axial pathway, (level V) distal airway.

ous airway levels, e.g., the ciliated cell being about 1 in 5 centrally, 2 in 3 peripherally.

At a given airway level, the pattern of glycoprotein within the secretory cells varies (*4, 18, 29, 30*). The epithelial glycoprotein is either neutral or acid (*16-18*): it is acid either because of sialylation or sulfation of the glycoprotein, and the sialic acid may or may not be susceptible to digestion by the enzyme sialidase. Sometimes within a single cell only one type of granule is identified, or there may be a mixture. A cell can be characterized by its predominant granule type and also by the amount of secretion, i.e., by the number of granules it includes. Regional differences in the normal pattern of glycoprotein distribution are also seen.

Cell division is another feature which has a basal level of activity (*6*). The oldest group of animals we have studied did not show a regional pattern, in that the percentage of cells incorporating ^{3}H-thymidine was similar at all airway levels. Since concentration of cells is less in peripheral airways, this means a slower replacement per unit area in small than in large airways. In the young animals, in male more than female, mitotic activity is more marked in the trachea than in peripheral airways. This sex difference was apparent in the young animals although male and female animals were gaining weight at the same rate.

Homeostasis then is maintained, on a regional basis, with respect to types of cell present, their proportion, their rate of mitosis and the pattern of intracellular glycoprotein identified histochemically.

Analysis of Mucus Secretion, Cellular Differentiation, and Their Control

The mucus glycoprotein comes from several cell types both within the submucosal glands and the surface epithelium; the mucus is then mixed with serum components. The structure of the airway wall changes with age and disease. Because of its complexity to understand airway secretion and its control, we have used a multidisciplinary approach in its study. Tissue studies of the normal human airway and of its modification in hypersecretory states offer an important reference. In organ culture systems of human airway behavior of the various cell types can be explored and the way their activity is controlled (*31, 32*). The total secretion can be analyzed as sputum and also material collected in organ culture (*11*). Environmental conditions can be altered in animal models. By organ culture of tissue obtained from such animals *in vivo* studies can be correlated with the *in vitro* (*33, 34*).

Our more recent organ culture studies of human epithelium show that secretory cells of the surface epithelium, at least of large airways, unlike the submucosal glands, are not stimulated to secrete by acetyl choline or its analogs. ^{3}H-Threonine transport into mucous and serous cells is largely ouabain-sensitive, ^{3}H-glucose only partially so. Postpulse addition of several agents has established that precursor uptake, glycoprotein synthesis and discharge, by mucous and serous cells, are not coupled events (*31*). In a series of experiments the effect of colchicine and cytochalasin B has been followed (*32*). Neither agent influences basal rate of secretion, of either mucous or serous cell. When incubated with the tissue and radioactive precursor, both reduce acetylcholine-stimulated secretion. It seems that the cytochalasin B-sensitive filament system must be intact for the discharge effect of a cholinergic agent.

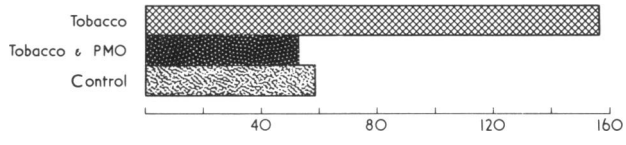

FIGURE 4. Mean number of secretory cells in 6 mm of rat tracheal epithelium after inhalation of tobacco smoke (tobacco), alone or with phenylmethyloxadiazole (tobacco + PMO), or after air alone (control).

Resetting the Homeostatic Mechanism

The homeostatic mechanism is reset to a different level by a variety of stimuli — irritants, infections, and drugs.

Irritants

The biological response of the epithelium is illustrated first by its reaction to tobacco smoke (*30, 33, 35*), with or without the addition of an anti-inflammatory agent (Fig. 4). Phenylmethyloxadiazole (PMO) is the agent illustrated although similar results have been produced by phenylbutazone. Rats were exposed to a smoke from cigarettes, delivered from a Wright auto-smoker over a period of 4 hr. In the first experiment described, this 4-hr daily exposure was repeated for 4 days a week for 6 weeks. The tobacco produced an increase in the concentration of secretory cells. Phenylmethyloxadiazole (PMO) prevented this in the extrapulmonary airway. Analysis of intracellular types of glycoprotein showed a shift in their pattern. In the trachea the shift is from a population of cells that contains predominantly neutral glycoprotein to one that is acid-producing. This shift occurs whether or not PMO is administered, that is, whether or not there is an increase in secretory cell number. From our studies we know that this shift can be detected within 24 hr, which makes it the most sensitive marker of change in airway epithelium.

Tobacco also increases mitotic activity. A burst of mitosis is seen 24 hr after starting exposure to tobacco smoke; PMO gives partial protection against this increase. The increase in secretory cells, particularly mucous cells, arises, in part, from conversion of undifferentiated cells to secretory cells, from new cells that appear as the result of mitosis and within the secretory cell population, by interconversion of serous or Clara cells to mucous cells. The interconversion is apparent from cell counts and from identification of intermediate cell forms (9). Electron-dense granules become mixed with electron-lucent granules, until, finally, all are electron-lucent and confluent. By light microscopy it is possible to identify increase in Alcian Blue staining granules. When a cell switches to secrete acid glycoprotein the AB-positive granules appear first at the apex of the cell. The "switch-on" of enzyme responsible for the attachment of sialic acid seems to start near the cell apex after the secretion is already packaged in its granules. The shift is first to sialylation and then to sulfation. When the latter occurs, the sulfated granules also appear first at the apex with sialylated granules being present nearer the nucleus.

On studying shorter exposure time, it emerged that this lability of the cell population, particularly of its secretory cells, is apparent after only one exposure to tobacco smoke (*5*) (Table 1). In this experiment the response to tobacco smoke was monitored at intervals up to 14 days. Animals were sacrificed 20 hr after the last exposure. Extrapulmonary and intrapulmonary airways showed different patterns of response. In the case of the trachea, one period of exposure to tobacco produced a discharge effect so that total secretory cell number was reduced. One exposure produced tolerance to the discharge effect, so that after two days' exposure the secretory cells had increased significantly above control values. In the intrapulmonary airways no discharge effect was seen: the one 4-hr exposure period significantly increased the secretory cell number above control values. Some further increase developed during the second week, when the concentration of secretory cells was virtually the same as that seen after six weeks' exposure.

In the trachea tolerance to exposure is quickly lost. One day's rest from exposure restores tolerance so that the next exposure produces a discharge effect. Adaptation to the environment is rapid and in a short space of time the maximal effect is produced. Although tolerance is quickly achieved it seems also

Table 1. Mean number of secretory cells in 3 mm of epithelium in control rats and after a single 4 hr period of inhalation of tobacco smoke.

	Level	Control	Tobacco
Extrapulmonary airways	I	34 ± 8.2	5 ± 1.9[a]
	II	41 ± 8.1	7 ± 0.9[a]
	III	64 ± 6.5	53 ± 4.6
Intrapulmonary airways	IV	27 ± 6.4	64 ± 7.4[b]
	V	6 ± 2.8	17 ± 5.3[a]
	VI	28 ± 6.4	29 ± 9.7
	VII	6 ± 2.0	7 ± 0.2

[a] $p < 0.01$.
[b] $p < 0.05$.

to be quickly lost. The homeostasis mechanism is reset for each of its features, secretory cell type and population, mitotic activity and glycoprotein pattern: a regional pattern is still apparent.

The anti-inflammatory agent PMO has modulated the response — offering protection from some aspects of the irritant, not from others; PMO given alone, by parenteral injection, causes increase in submucosal gland size. *In vitro* study of submucosal gland from these animals shows reduced precursor uptake, as well as decreased discharge. The animals who had received tobacco plus PMO showed normal secretory rate, whereas tobacco alone produces an increase in secretory rate.

Drugs: β-Adrenergic Agents, Isoproterenol, and Salbutamol

We have shown that isoproterenol causes hypertrophy of the bronchial submucosal gland and also increases surface epithelial secretory cell number, even under germ-free conditions (*10, 36-38*). It is the secretory cells producing acid glycoprotein that are particularly increased in the surface epithelium (Fig. 5). In a recent experiment we have extended these studies to follow the effect of isoproterenol over shorter times and at various airway levels and to compare it with that of salbutamol (a nonselective β and a selective β_2 agonist, respectively) (Fig. 6).

The two drugs produce increase in secretory cell number, but with striking regional differences. At all levels both drugs cause a shift to acid glycoprotein production and this, whether or not secretory cell number is increased. With respect to secretory cell number the drugs show different regional specificity. Isoproterenol causes increase in secretory cell number at all levels save the midtrachea (so this pattern is not based on the distribution of nerves). Salbutamol only produces an increase at the most proximal tracheal level and the most distal part of the axial pathway of left lung. At each level the salbutamol effect is less marked than the isoproterenol. The effect can be detected after one injection of isoproterenol. These studies also show that the lability of the epithelial cell population is striking and rapid, and the maximal response is quickly achieved.

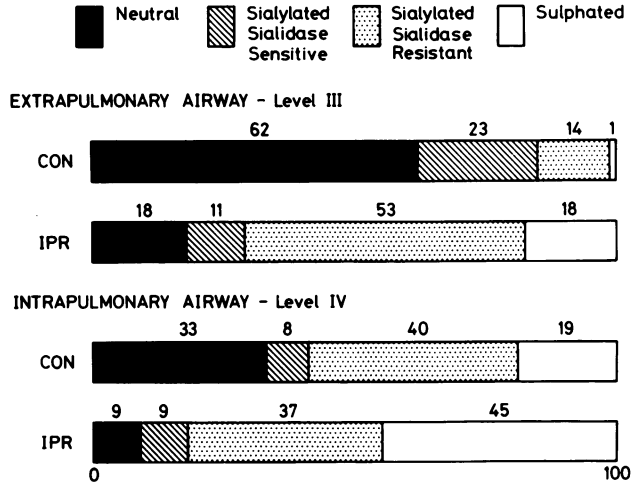

FIGURE 5. Percentage of secretory cells containing four types of glycoproteins in control (CON) and isoproterenol-treated (IPR) rats. Level III = distal trachea, level IV = proximal region of intrapulmonary axial pathway.

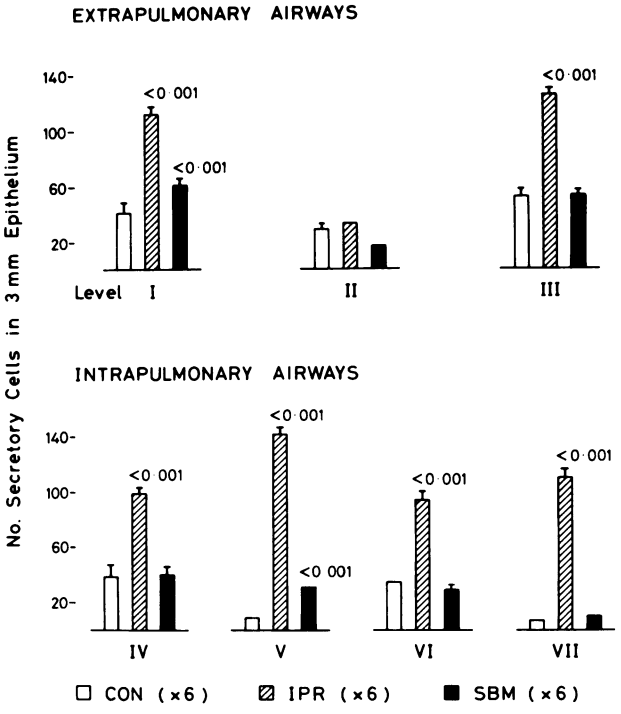

FIGURE 6. Mean number of secretory cells in 3 mm of airway epithelium of control (CON), isoproterenol-treated (IPR), or salbutamol-treated (SBM) rats at seven levels: (levels I-III) – proximal, mid, and distal trachea with main bronchi; (levels IV and V) proximal and distal region of main intrapulmonary axial pathway; (levels VI and VII) lateral branches 6x injections of drug or normal saline (controls).

Recovery

After ceasing administration of the drug, recovery has been followed; here also major regional differences are seen (Fig. 7). In the trachea the secretory cell concentration returns virtually to normal within a week or so. In other regions differences persist. In the main bronchus, even 12 weeks later, the secretory cell number has not returned to normal. At this level there is a stage when secretory granules are

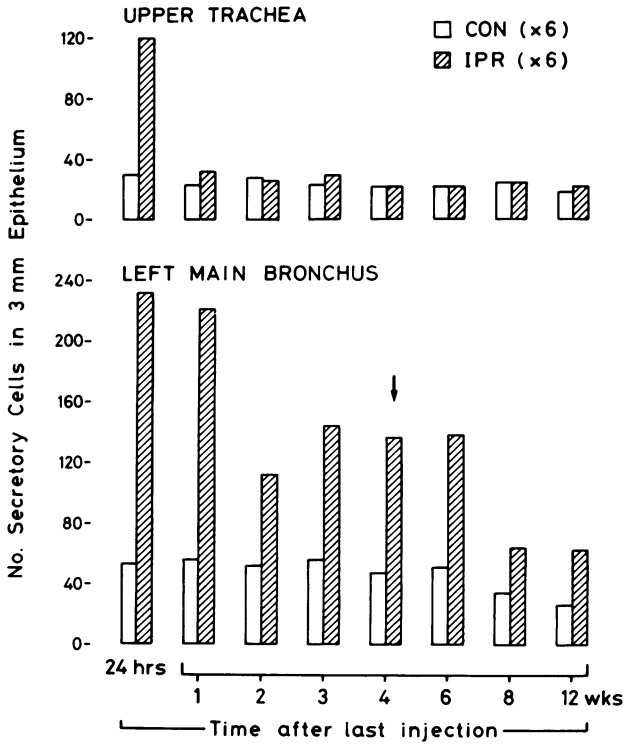

FIGURE 7. Mean number of secretory cells in 3 mm of epithelium in the upper trachea and left main bronchus of control (CON) and isoproterenol-treated (IPR) rats after single daily injections on 6 consecutive days of either normal saline (controls) or isoproterenol (10 mg/100 g body weight). Number of cells present 24 hr after the last injection and after periods of 1 to 12 weeks recovery.

seen as ghost structures and an irregular mixture of granules of acid and neutral glycoprotein is seen, different from the orderly way in which a "switch-on" to acid glycoprotein occurs. It seems that the airway does not become tolerant of the stimulus, the tissue does not adapt permanently, since on withdrawal of the isoproterenol, the airway reverts to normal. During recovery a greater variance is seen between individual animals in the same group than during adaptation to the drug. This is particularly true of surface epithelium. In the submucosal gland also, hypertrophy is produced by isoproterenol and has been shown to revert to normal within six weeks (*36*).

It seems that this pattern of response reflects regional differences in sensitivity, based perhaps on regional differences in the nature and concentration of receptors. Since these drugs mimic naturally occurring agents, it could be that the adrenergic system, β and probably α, is part of the normal control system. Either way the use of the appropriate agonists and antagonists offers a way to modulate secretion, certainly experimentally, and perhaps clinically also.

Intra- and Extracellular Conditions

The histochemical studies of the glycoprotein within the cell are of special significance in showing the shift to increased acidification of the oligosaccharide side chains, with relative increase in sulfated forms (Fig. 5) and, within the granules that are only sialylated, to those in which more of the sialic acid is resistant to sialidase. Understanding of the composition and structure of bronchial mucus glycoproteins (as of other epithelial glycoproteins, e.g., gastric and cervical) is an actively expanding field both as regards the polypeptide core and the oligosaccharide side chains (*20, 23, 39*).

The histochemical shift seen in states of hypersecretion is associated with the biochemical finding of increased concentration of both *N*-acetylneuraminic acid (*syn*-sialic acid) and sulfate in the oligosaccharide fraction. Sometimes both occur on the same chain. The largest chains are usually the most heavily sulfated. It seems that whereas sialic acid is a terminal sugar, sulfation permits further glycosylation.

Animal studies reveal the striking lability of the intact epithelium both as regards its cell mix and its glycoprotein product. These adjustments occur in the airway cells whether or not increased mitotic activity is also part of the response to a given stimulus. The speed of adaptation and its nature raise questions as to the mechanisms of homeostasis responsible for stability in such a complex population of cells that yet shows such striking regional variation. These questions then lead to those concerned with the basis of the resetting of this regulation by irritants and drugs. Understanding of these changes can be expected to indicate what is needed to control the features of adaptation that are undesirable clinically.

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